Continental J. Tropical Medicine 5: 10 - 14, 2011 ISSN: 2141 - 4167

http://www.wiloludjournal.com
Printed in Nigeria

AN INVESTIGATION INTO THE ANTI-MALARIA PROPERTY OF ETHANOLIC EXTRACT OF THE LEAVES OF *GONGRONEMA LATIFOLIUM* ON ARTESUNATE SENSITIVE *P. BERGHEI* INFECTED ALBINO MICE

Esume C.O, Emudainohwo J.O.T, Opajobi A.O, Osifo I.M and Onyemekeih U.R
College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria

ABSTRACT
Objective : This study investigated the anti-malaria properties of ethanolic extract of the leaves of *G. Latifolium* on artesunate sensitive *P. berghei* infected albino mice.

Methods: Peter's 4 days suppressive test was carried out on 25 mice sub-grouped into 5 treatment groups with 5 mice per group. 3 of the group received 3 different doses of the extract (150mg/kg/day, 300mg/kg/day and 45omg/kg/day) for four days, the other groups being the control and standard group received 5ml/kg/day and 5mg/kg/day of distilled water and artesunate respectively. Results The percentage chemo suppression observed was as high as 87.07%, 97.65% and 99.42% for 150mg/kg/day, 300mg/kg/day and 450mg/kg/day respectively. The artesunate treated mice showed 98.22% chemo suppression.

Conclusion: The result obtained from the study showed strong anti-malaria ($P< 0.05$) effect that is comparable if not equivalent to artesunate.

KEYWORDS: Albino mice, anti-malaria, *G latifolium*, artesunate, *plasmodium Berghei*.

INTRODUCTION

Malaria has continued to be the most devastating human parasitic infection in the world, with about 200 million people at risk of infection (Giles, 1991). It is said to be responsible for over 2 million deaths each year, and a leading cause of death in children in countries where the disease is endemic. These facts, makes malaria one of the most important health problem in tropical Africa, including Nigeria.

Much of the mortality from the disease is caused by infection with *plasmodium falciparium* which have been reported to pose the greatest risk to non-immune individuals and children less than 5 years of age (Hardman and Limbird, 2001)

The present increasing prevalence of multi drug resistant strains of the parasite, with an attendant rise in failure of treatment with several of the available drugs that used to be effective have made it necessary to keep looking for new sources of safer and effective drugs.

In many parts of the world, malaria is still being treated by orthodox and traditional medicine (Okpako, 1999). Plant has been the source of important compounds of some presently available useful anti-malaria drug like quinine and artemisinine (Li and Wu, 2003). The crude extract of cinchona tree bark and leaves of *Artemisia annuus* were used successfully in the treatment of malaria infection (Guerra 1997).

Gongronema latifolium, a perennial climber crop, native of the humid tropic of South Eastern Nigeria (Okafor, 1989), known locally among the Efik, Ibibio and Igbo speaking communities of Nigeria at Utazi, could have some protective effect against certain hepatocallular injury. The Efiks and the Quas in Calabar, use *G. latifolium*, crude leaf extract in the treatment of malaria, diabetes, hypertension and as a laxative. Also, it is used as vegetable (Morebise *et al*., 2002). Based on the fact that *G. latifolium* is being used by the Efiks and Quas in Calabar, for the treatment of malaria, the findings from the study would either corroborate or contradict the peoples acclaimed health benefit or might reveal unrecognized or concealed toxic outcomes of the use of *G. latifolium*.

MATERIALS AND METHODS

The research was carried out in the Department of Pharmacology and Therapentics laboratory, Delta State University, Abraka. Fresh leaves of *G. latifolium* was collected from an uncultivated farm land in Agbor, Ika South Local Government Area of Delta State, and authenticated by a taxonomist in Botany Department, Delta State University, Abraka.

Preparation of Plant Extract

The fresh leaves of *G. latifolium* (utazi) were sun dried for a period of five days. The dried leaves were blended with an electric blender, into fine powder. 200g of the blended leaves was soaked in 1000ml (ratio1:5) of absolute ethanol for 48 hours, and was filtered, using Watman filter paper. The filtrate obtained was evaporated, using an evaporator. The powdered extract obtained, was transferred into a reagent bottle and stored in a refrigerator.

The stock solution was prepared by dissolving 10g of the powdered extract in 100 ml (ratio 1:10) of distilled water, to give a stock concentration of 0.10g/ml.

Experimental Animals

25 healthy male albino mice (20-32.3g) obtained from animal house, College of Health Science, Delta State University Abraka, were housed in a mosquito-net screened cage and acclimatized for a period of 14 days. They were fed with growers mash and water.

Parasite Inoculation

Artesunate sensitive *P. berghei* strain maintained in mice were obtained from National institute of medical Research (NIMR), Yaba, Lagos State, Nigeria.

Each mouse was inoculated on day 1; intraperitoneally, with about 0.2ml of infected blood containing *P. berghei* parasitized red blood cell obtained from a donor mouse having parasite load of $1x10^3$ parasitaemia per high power field.

Evaluation of malaria parasite

The mice were infected on day 1, with infected erythrocyte intraperitoneally. After 72 hours, their parasite load was evaluated by preparing thick blood film, from blood collected by transection of the distal end of their tail, with a pair of scissors after cleaning with methylated spirit cotton swab. Three drops of blood were allowed to drop on a slide and with the smooth angle edge of another clean slide used as a stirrer, a thick smear film was made.

The thick smear of correct thickness is one through which new print is barely visible. The films were air dried, fixed in methanol for 30 seconds and stained with 10% Giemsa for 20 minute. The slides were rinsed carefully and thoroughly under running tap water and left, to stand in an upright position to dry.

Prepared slide were viewed under x50 objective (oil immersion) light microscope with special ocular condenser sufficiently close to give a good contrast. Parasite (pinkish in color) made up of cytoplasm and chromatid dot (sand-like dots) were seen in the area of the Giemsa stain while the white blood cell was bluish in color. The parasite was counted in x50 high power fields and parasite count recorded.

Drug Administration

All drugs were administered orally and based on the weight of each mouse.

Evaluation of Blood Schizonticidal Activity in Established Infection(Suppressive Test)

Peter's 4 day suppressive test against *P. berghei* infection in mice was employed (Peter's 1970).

The mice were divided into five groups (n=5) of three mice each. The first 3 groups were administered 150, 300 and 450mg/kg/day dose respectively of the extract for four consecutive days, while the fourth group was administered artesunate 5mg/kg/day and the fifth group was administered equivalent volume(5ml/kg/day) of distilled water (control) for four consecutive days.

On the fifth day, blood smear was prepared and the number of parasite was counted in x50 high power field.

The average percentage suppression of parasitaemia was calculated of control as shown bellow.

Average of suppression = average % parasitaemia in control groups ─ average of parasitaemia in treated groups

x 100 / Average % parasitaemia in control group.

That is: $\frac{(\text{control mean} - \text{dose mean})}{\text{Control mean}} \times \frac{100}{1}$

The mean were calculated as mean ± standard error of mean (SEM) where SEM = $\frac{\text{Standard Deviation}}{\sqrt{N}}$

RESULTS

Of the 200g weight of powered *G. Latifolium*, 10g was extracted and used for the treatment test. Based on the lethal concentration, dose treatments of up to 450mg/ kg were prepared for the suppressive Test. The results are as presented in Table 1.

TABLE 1: Summary of Suppressive Test.

Test Group	Dose	Pre-treatment parasite count	Post-treatment parasite count	% Suppression
Group 1 (control)	5ml/kg/day distilled water	31±2.65	56.67±3.05^	0
Group 2	150mg/kg/day extract	28.67±2.73*	7.33±1.76^	87.07
Group 3	300mg/kg/day extract	37±2.65*	1.33±0.33^	97.65
Group 4	450mg/kg/day extract	26±1.83*	0.33±0.33^	99.42
Group 5 (Standard)	5mg/kg/day Artesunate	38.33±3.67*	0.67±0.33^	98.22

* P>0.05, not statistically significant compared to control

^P<0.05, is statistically significant compared to control

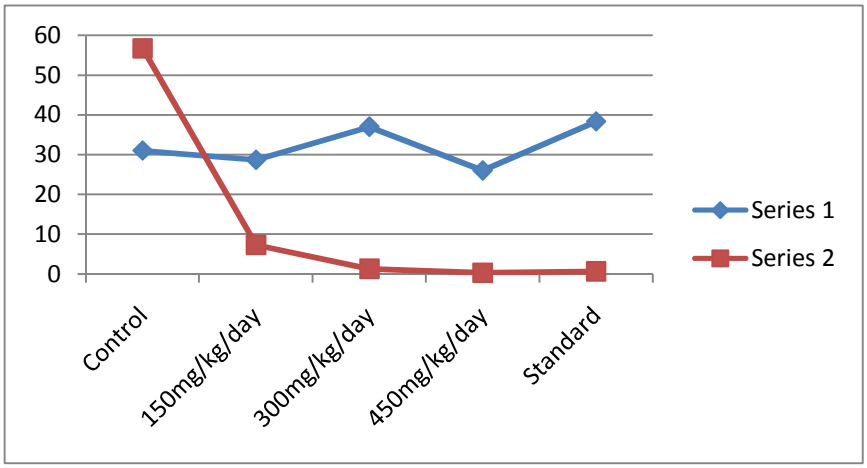

Fig. 1: Anti-malarial property of *G. latifolium* on artesunate-sensitive *Plasmodium berghei* strains.
Series 1 – Pre-treatment
Series 2 – Post-treatment

DISCUSSION

The study, an investigation into the ant-malarial property of *G.latifolium* revealed that the plant ethanolic extract has anti-malarial property.

The main active ingredient for this effect is not known. Although, the presence of glycosides, alkaloids, saponnins, tannins, flavonoids and carbohydrates (Nwanjo *et al*., 2006) has been implicated in anti-plasmodial activities and as such, anti-plasmodial activity observed in this study might be due to either a single additive or synergistic action of these compounds (Okwu,2004).

The percentage chemo-suppression is about 87.07, 97.65, 99.42, and 98.22 for 150mg/kg/day, 300mg/kg/day, 450mg/kg/day and artesunate-treated groups respectively. With respect to change in parasitaemia between pre-treatment and post-treatment of each group, it was observed that 150mg/kg/day, 300mg/kg/day, 450mg/kg/day and artesunate-treated groups showed significant decrease ($p<0.05$) in mean parasitaemia, while the control group that received distilled water showed a marked increase in mean parasitaemia level from 31±2.65 to 56.67±3.05 (Fig1). From the results obtained from Table 1 above, the post-treatment parasitaemia level decreased significantly in the groups treated with the extract of *G. latifolium* (150mg/kg/day, 300mg/kg/day, 450mg/kg/day), when compared to the control that received distilled water ($P<0.05$). The effect showed no significant difference from Artesunate-treated group ($P>0.05$).

These results validate its use in folk medicine by the Efik and Quas in Calabar for the treatment of malaria.

CONCLUSION

This study showed that the ethanolic extract of *G. latifolium* has significant anti-malarial effect that is comparable, if not equivalent, to that of artesunate against artesunate-sensitive *Plasmodium Berghei* strains. Nevertheless, the limitation of this study was that the extract was not tested on resistant strains of the parasite which is the frontline of anti-malarial research. Also the mechanism of action is yet to be elucidated. Meanwhile, it is clear that this plant can make a major contribution in the control and treatment of malaria in artesunate-sensitive strains.

REFERENCES

Giles HM (1991). Management of severe and complicated malaria. WHO, Geneva.

Guerra P (1977). Plants and their active constituents. *Cinenc Cult* (Brazil) 29:599- 600

Okafor J.C. (2005). Conservation *and uses of traditional woody forest species in southeastern Nigeria*. Fame Agricultural Centre,Enugu,Nigeria.

Hardman J.G. and Limbird L.E (2001). *Drugs Used in Chemotherapy of Malaria*. In Mc Graw-Hill eds. Goodman and Gilman. The Pharmacological Basis of Therapeuctics, 10th ed. USA. PP 1069

Li .Y, Wu. Y.L. (2003). "An Over four millennium Story Behind qinghaosu (Artenisinin)—a Fantastic Anti-Malaria Drug from a Traditional Chinese herb" *Curr Med Chem*. 21: 2197-2230

Morebise O., Faunson M.A, (1998). Anti-Microbial and Phyto-toxic Activities of Saponin extracts from Two Nigerian Edible Medicinal Plants. *Biokemistri* 8 (2) PP 69-77

Morebise ,Fafunso M.A, Makinde J.M,Olajide O.A, Awe E.O.(2002) Anti inflammatory property of the leaves of Gongronema latifolium . *Phytother Res*. pp75-77

Nwanjo,H.U and Alumanah, E.O(2005). Effect of aqueous extract of G.latifolium leaf on some indices of liver functions in rats. *Global J.Med.Sci*.4(1);29-32

Okwu D.E.(2004):phytochemical and vitamins content of indigenous species of South Eastern Nigeria. *Journal of Suitable Agriculture Environment.* 6 (1) :30-31

Received for Publication: 13/03/2011
Accepted for Publication: 28/04/2011

Corresponding Author:
Esume C.O.
College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria